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Promotion of cone production in potted black-spruce grafts, using gibberellins, heat-treatment and root-pruning

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ABSTRACT

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Potted 3-year-old grafts of black spruce [*Picea mariana* (Mill.) B.S.P.] at different stages of lateral shoot elongation were sprayed with different concentrations of gibberellin $A_{4/7}$ for a total of six weeks in a heated greenhouse to enhance seed-cone production. Grafts sprayed during the period of lateral shoot elongation produced significantly more seed cones but not pollen cones, while spraying initiated after the elongation did not promote any cone production. Increasing gibberellin did not increase production, but increased graft mortality when spraying was done during the period of rapid shoot elongation. Cone production was highest and mortality lowest with 200 mg l^{-1} gibberellin $A_{4/7}$ spraying that was initiated in the middle of rapid shoot elongation. In a second experiment, potted 5-year-old grafts with or without root pruning were placed either outdoors or in a heated greenhouse during the period of lateral shoot elongation. Heat-treatment during the period of late slow shoot elongation had a positive effect on pollen- and seed-cone production in 5-year-old grafts without root-pruning. A combination of heat-treatment and root-pruning appeared to have an adverse effect on cone production. Pollen viability was not affected by the treatments.

INTRODUCTION

Gibberellin $A_{4/7}$ ($GA_{4/7}$) application, heat-treatment and root-pruning, alone or in combination, are effective treatments for promoting cone production when they are applied to spruces (*Picea* spp.) during the period of shoot elongation. Tompsett and Fletcher (1979) and Ross (1985) reported that $GA_{4/7}$ application significantly increased cone production on potted grafts. Spruces subjected to elevated temperatures in a polyhouse exhibited greater cone production (Tompsett and Fletcher, 1977, 1979; Luukkanen, 1980; Chalupka, 1981; Philipson, 1983; Ross, 1985, 1988a). Effectiveness of heat and drought treatment was further enhanced by $GA_{4/7}$ spraying (Ross, 1988a). These types of reproductive induction procedures in a greenhouse

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can facilitate breeding processes that are often hampered by poor weather and remoteness of a seed orchard. Pharis and Ross (1986) have discussed the potential of this technology for producing seed for progeny testing and genetically improved seed for reforestation.

$GA_{4/7}$ spraying of black spruce [*P. mariana* (Mill.) B.S.P.] grafts growing in a seed orchard, during the period of lateral shoot elongation, was found to be effective in promoting seed cones (Ho, 1988b). Pharis et al. (1986) reported that weekly spraying of $GA_{4/7}$ onto branches or injection once every 2 weeks into the base of girdled branches of white spruce (*P. glauca*) from mid-July (after shoot elongation) to late September in southern Ontario significantly increased seed cones not pollen cones. Effectiveness of spraying in the summer would facilitate operational application after the spring rush. The present study examined the feasibility of late application to potted black-spruce growing in a heated greenhouse, and investigated the effect of root-pruning and heat-treatment during the period of lateral-shoot elongation on cone production.

MATERIALS AND METHODS

Scions from black-spruce plus trees were grafted onto white-spruce seedling rootstocks in 1981 and 1983, and experiments were carried out in 1986. Thirty-two ramets each of 15 clones were grafted and established in 4.5-l pots in 1983 (3-year-old grafts), and 26 ramets each of 16 other clones in 9-l pots in 1981 (5-year-old grafts). The soil mixture consisted of peat, perlite and sand (5:4:1 *v*:*v*:*v*). The average height for 3-year-old grafts was 0.7 m, and for 5-year-old grafts, 1.2 m.

While in a heated greenhouse or outdoors, grafts were watered as required and fertilized (balanced fertilizer N:P:K 20:20:20 with minor elements) once a week. The greenhouse was maintained at 30°C day and 20°C night, with a photoperiod of 18 h. On a bright, sunny day in July and August, the temperature occasionally reached 38°C for a short period of time around noon.

$GA_{4/7}$-spraying/heat-treatments

Four spraying concentrations were factored with four spraying periods in the experiment. Roots of 3-year-old grafts were deeply cut at four places around the circumference of the root ball to sever the circular roots before repotting into 15-l pots in April 1986.

The treatment schedule was based on bud-burst and lateral-shoot elongation, which was divided into early-slow, rapid, and late-slow shoot elongation (Ross, 1985). In mid-May, about a week after lateral vegetative bud burst, eight ramets each of 15 clones were placed in a heated greenhouse where two ramets of each clone were sprayed weekly for a total of six weeks with 0, 200, 400 and 800 mg $GA_{4/7}$ l^{-1} solution each containing 15% ethanol and 0.05%

Tween 20 surfactant. Foliage of the grafts was thoroughly wetted with a fine mist sprayer. Three weeks later at the late-rapid shoot elongation, the second set of 120 ramets was placed in the greenhouse for the same treatments. Thereafter, every three weeks another set was brought in for treatment, and the last two treatments were carried out after the end of lateral-shoot elongation. Each set was moved outdoors to a holding area a week after the last spray.

Elongation of terminal shoots of the first-order lateral branches was monitored weekly on two ramets each of the 15 clones per period. Data on seed-cone production were collected in the year following treatments. The numbers of grafts producing pollen cones were counted, and pollen was extracted from ripe pollen cones from each producing tree. Pollen viability was tested for each producing ramet by evenly spreading pollen grains onto each of two petri dishes containing 0.6% agar and 5% sucrose, and incubating the dishes at room temperature for 48 h. A total of 100 pollen grains was counted from each dish. A pollen grain was considered germinated when a pollen tube had elongated by a length equal to at least one-half of the length of a pollen grain.

Root-pruning and heat-treatments

Roots of one half of the 5-year-old grafts were cut to sever the circular roots, and repotted as described above. The other half were root-pruned (about 50% of active roots removed), cleaned of soil, and repotted in 15-l pots.

Four periods of heat-treatment were factored with two root-pruning treatments. Two ramets each of 10 clones were used for each treatment. About a week after lateral vegetative bud-burst, one set of 40 root-pruned and 40 non-pruned grafts was placed in the greenhouse for five weeks and the other set for nine weeks before being transferred outdoors. The third set was moved into the greenhouse five weeks after bud-burst (late-slow shoot elongation), and the fourth set was left outdoors as controls.

Monitoring of lateral-shoot elongation, data collection, pollen extraction, and pollen germination were as described in the section $GA_{4/7}$ spraying/heat-treatments.

Data analysis

A structured design was used for both studies. Because of the mortality during the course of each study, data on seed-cone production in the $GA_{4/7}$-spraying/heat-treatment study (3-year-old grafts) cannot be normalized with a transformation, so a nonparametric procedure was used for the analysis based on the classifications of producers and non-producers (Steel and Torrie, 1980). In the root-pruning/heat-treatment study (5-year-old grafts), data on seed- and pollen-cone production per surviving tree were normalized with

a square-root transformation, and analysis of variance was carried out after the transformation. In pollen germination, an arcsin transformation was carried out before analysis of variance.

RESULTS AND DISCUSSION

$Ga_{4/7}$-spraying/heat-treatment

Elongation of the terminal shoot of the first-order lateral branch had about one week of early-slow shoot elongation (7 May–13 May) followed by four weeks of rapid elongation (14 May–10 June) and then about one week of late-slow elongation (11–18 June). Shoot growth was completed by 20 June. Marquard and Hanover (1984) had a similar report for the pattern and length of time in shoot elongation for white spruce in Michigan. Ross (1985) indicated a total of nine weeks in shoot elongation in Engelmann spruce (*P. engelmannii*) in British Columbia; rapid elongation spanned five weeks, and early- and late-slow elongation spanned a two-week period each. The pattern of lateral-shoot elongation in black spruce was similar for controls and treated grafts, and the spraying had no effect on the lateral-shoot elongation of the current year's growth (9.5 cm) (Ross and Pharis, 1976).

$GA_{4/7}$-spraying/heat-treatment, and heat alone, significantly ($P \leq 0.05$) promoted seed-cone production on the 3-year-old black-spruce grafts when the six-week treatment was initiated a week after lateral vegetative bud-burst or in the middle of rapid shoot elongation, but not later (Table 1). Increase in $GA_{4/7}$ concentration did not significantly ($P \leq 0.05$) improve seed-cone production. The results did not confirm that weekly spraying from July through September was effective in inducing cones (Pharis et al., 1986), but confirmed that spraying was effective when applied during lateral-shoot elongation (Marquard and Hanover, 1984; Ross, 1985). Effectiveness was further enhanced when $GA_{4/7}$ was applied with heat treatment (Tompsett and Fletcher, 1979; Philipson, 1983; Ross, 1985, 1988a). This is further substantiated by the reports that $GA_{4/7}$ has a morphologic effect on bud-type differentiation (Ross, 1985) and bud-type differentiation occurs soon after the shoot elongation (Marquard and Hanover, 1984). Spraying during and after the bud-type differentiation could not change the course of differentiation since biochemical differentiation precedes morphological differentiation (Ho, 1988b).

$GA_{4/7}$-spraying did not promote pollen-cone production in black spruce (Table 1). Only one or two grafts sprayed with $GA_{4/7}$ in each spraying period produced pollen cones, while as many as seven grafts in controls produced pollen cones. Marquard and Hanover (1985) reported similarly that no pollen cones were produced in white spruce sprayed with $GA_{4/7}$. However, most studies found that $GA_{4/7}$ was a good promoter of pollen-cone production in

TABLE 1

Average number of 1987 seed cones per graft (±SE), percentage of mortality and average percentage of pollen germination (±SE) in potted 3-year-old black-spruce grafts which were sprayed at different stages of lateral shoot elongation with 0, 200, 400 or 800 mg l^{-1} $GA_{4/7}$ for six weeks in 1986

Spray periods	Shoot elongation	$GA_{4/7}$ concentration (mg l^{-1})			
		0	200	400	800
Average number seed cones per graft					
13 May–24 June	rapid	14.5±8.1	31.6±8.6	41.7±14.9	28.2±13.3
3 June–15 July	rapid	9.9±4.8	38.1±8.3	34.7±5.9	22.5±5.2
25 June–5 August	end	0.1±0.1	0.3±0.1	0.5±0.2	0.1±0.1
15 July–26 August	end	0.1±0.1	0.2±0.2	0.0±0.0	0.3±0.2
Percentage mortality					
13 May–24 June	rapid	6.7	43.3	56.7	60.0
4 June–15 July	rapid	3.3	13.3	20.0	23.3
25 June–5 August	end	0	6.7	10.0	10.0
15 July–26 August	end	3.3	0	0	6.7
Average percentage of pollen germination	% of grafts producing pollen cones				
13 May–24 June	4	70.0±5.0	–	–	89.0±1.0*
3 June–15 July	11	59.3±7.2	84.0±0*	83.0±5.8	88.0±0*
25 June–5 August	6	78.3±3.0	74.3±4.8	17.0±1.0*	66.0±0*
15 July–26 August	3	86.0±0*	85.5±1.5	–	–

*only one graft producing pollen cones.

spruces (Tompsett and Fletcher, 1979; Cecich, 1985; Philipson, 1985; Ross, 1985).

The effect of foliar $GA_{4/7}$-spraying on cone production appears to be dependent on concentration, timing, frequency, duration, tree age, genotype and species (Marquard and Hanover, 1984, 1985). In black spruce, Hall (1986) reported that monthly spraying of $GA_{4/7}$ at 200 and 800 mg l^{-1} on 20-year-old trees was not effective. However, Ho (1988b) found that $GA_{4/7}$ at 400 mg l^{-1} was effective in weekly spraying on 14-year-old grafts during the period of lateral-shoot elongation. Based on cone crops and mortality of 3-year-old grafts, 200 mg $GA_{4/7}$ l^{-1} appeared to be best for weekly spraying for six weeks, initiated in the middle of rapid lateral-shoot elongation of potted black spruce (Table 1).

The mortality of potted grafts increased with the increasing concentration of $GA_{4/7}$, but occurred in the periods when spraying was most effective in promoting seed-cone production (Table 1). Droopy branches were the first

injury symptom followed by the drying of needles and needle cast with some discoloration from green to yellow (Ho, 1988a). The cause of death is not known, but excessive exogenous $GA_{4/7}$ might have resulted in stress or have modified metabolic processes. It is unlikely that the mortality resulted from the surfactant (Tween 20) application, because grafts sprayed with the solution without $GA_{4/7}$ had little or no mortality while those sprayed with the solution containing $GA_{4/7}$ had a mortality rate ranging from 13 to 60%, depending on the $GA_{4/7}$ concentration and the timing of spraying. However, Ross (1988a) attributed the death of $GA_{4/7}$-treated Engelmann spruce to surfactant (Aromox C-12/W) phytotoxicity.

Pollen viability of black spruce did not appear to be adversely affected by $GA_{4/7}$-spraying (Table 1). A few grafts produced some pale-red pollen cones in addition to bright- and dark-red cones, and these shed either no pollen or little pollen which was often low in viability.

Root-pruning/heat-treatment

Growth patterns in both bud-burst and shoot elongation of 5-year-old grafts were similar to the 3-year-old grafts except that the former were one week behind in bud-burst. Root pruning did not affect the seasonal pattern of shoot elongation, but lateral shoots of root-pruned grafts attained a final length only 60% of that of nonpruned grafts (11.5 cm). Marquard and Hanover (1985) similarly reported that a significant reduction in terminal-shoot growth was observed in white spruce. This could have resulted from the loss of some roots and nutrient reserves in the roots, and diversion of nutrients from the shoots for the formation of new roots.

Root-pruning did not significantly ($P \leq 0.05$) affect seed-cone production. Nonpruned grafts which were heat-treated in the period of late-slow shoot elongation produced about twice the number (98 and 127 cones per graft) of those which were growing outdoors (53 cones per graft). No significant difference was found between heat and outdoor treatment in root-pruned grafts (Table 2). In the analysis of variance, clonal variation was significant and accounted for 47% of the variance; the interaction between heat and pruning treatment (23%) was significant, indicating that the factors were not independent. The results from grafts without root-pruning confirmed Ross' report (1985) on cone induction by high temperature. Heat-treatment coupled with root-pruning appeared to have a negative effect in black spruce, but the coupling was necessary for Sitka spruce (*P. sitchensis*) (Philipson, 1983) and white spruce (marquard and Hanover, 1985; Ross, 1988a).

Root-pruning significantly ($P \leq 0.05$) reduced pollen-cone production and the number of producing grafts (Table 2). The treatments accounted for 64% of the variation, and clonal variation was significant and accounted for 21% of the variation. No significant difference was found in root-pruned grafts

TABLE 2

Average number of 1987 seed and pollen cones per graft (± SE), percentage of grafts producing pollen cones, and average percentage of pollen germination (± SE) in 5-year-old black-spruce grafts which were or were not root-pruned before heat treatment at different stages of lateral shoot elongation

Root pruning	Treatment periods			
	Outdoor	Indoor		
		21 May–25 June	18 June–23 July	21 May–23 July
Average number of seed cones per graft				
Without	53.0 ± 19.8^{ab}	74.2 ± 17.5^{ab}	97.7 ± 25.5^{ab}	126.6 ± 23.2^{b}
With	115.9 ± 27.1^{a}	56.4 ± 11.9^{a}	59.6 ± 13.5^{a}	65.7 ± 16.0^{a}
Average number of pollen cones per graft				
Without	113.4 ± 52.5^{ab}	58.7 ± 15.6^{a}	103.6 ± 26.0^{ab}	185.1 ± 47.7^{b}
With	22.1 ± 15.8^{a}	24.9 ± 8.6^{a}	40.2 ± 14.0^{a}	40.6 ± 27.0^{a}
Percentage of grafts producing pollen cones				
Without	100	84.2	95.0	85.0
With	41.2	77.8	75.0	76.9
Average percentage of pollen germination				
Without	80.3 ± 2.7^{a}	70.8 ± 3.7^{a}	73.1 ± 3.3^{a}	72.1 ± 3.7^{a}
With	71.3 ± 2.4^{a}	80.3 ± 2.1^{a}	73.2 ± 4.8^{a}	80.3 ± 3.4^{a}

*Means within a row followed by the same letter do not differ significantly at the 5% level based on Scheffe's test.

growing outdoors and indoors, while nonpruned grafts produced the least pollen cones (59 cones per graft) when treatment was completed at the end of shoot elongation. The combination of heat- and root-pruning treatment appeared to have an adverse effect in black spruce. Heat-treatment alone was reported to be responsible for increase in pollen cones in Sitka spruce (Tompsett and Fletcher, 1979; Philipson, 1983), while the combination was necessary for profuse cone production in Engelmann spruce (Ross, 1988a).

Root-pruning did not have much effect on the survival of potted grafts. However, prolonged heat-treatment increased the mortality of root-pruned grafts (35%). Grafts produced mostly bright- and dark-red pollen cones, but a few produced some pale-red cones. Pollen viability did not appear to be affected by root-pruning and/or heat-treatment (Table 2). No significant differences ($P \leq 0.05$) in pollen viability were found between grafts without and with root-pruning among the four treatment periods. However, pollen viability was relatively low, ranging from 67 to 80%, compared with the viability of pollen (95%; own unpublished data, 1987) collected from orchard trees at the time of shedding. Although there was no published data on the viability of pollen from induced pollen cones in spruces, Ross (1988b) indicated that necrosis of pollen cones after forcing early pollen shedding in Engelmann spruce could have resulted from pycnosis of reproductive cells.

Black spruce responded to $GA_{4/7}$-spraying during the period of rapid shoot

elongation by producing seed cones, while heat-treatment during the period of late-slow shoot elongation tended to induce pollen cones. In a breeding program, it would be practical to apply both techniques to induce seed cones on trees from $GA_{4/7}$-sprayed trees and pollen cones on trees in a heated greenhouse, thus ensuring a pollen supply for controlled pollination.

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